INVESTIGATION OF THE EFFECT OF STINGING NETTLE EXTRACT ON NEUROLOGICAL ALTERATIONS DURING COMORBIDITY OF DEPRESSION AND TYPE 2 DIABETES

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Abstract

Clinically, depression and diabetes are co-morbid. Diabetes makes the symptoms of depression worse. Depression reduces overall physical and mental health, not only by increasing the risk for diabetes but making diabetic symptoms worse. Both depression and diabetes are the risk factor for cognitive impairment. Cholinergic system, autophagy (ATG), insulin signaling pathway and sonic hedgehog (Shh) pathway are involved in many regulatory processes, including learning and memory. Stinging nettle (*Urtica dioica*, UD) extract has been claimed for its beneficial effects against depression, diabetes and cognition. The present study was performed to evaluate whether chronic unpredictable mild stress (CUMS) or diabetes mediated cognitive deficit is associated with dysfunction in cholinergic system, ATG, insulin signaling and Shh pathway. In addition, standardized UD extract was used to evaluate its effect on CUMS or diabetes mediated neuronal dysfunction. Rosiglitazone, fluoxetine and St. John's wort were used as standard drugs for comparison. CUMS (3 weeks) and multiple dose of streptozotocin (STZ) (50 mg/kg, i.p. for 5 consecutive days) resulted in depressive like behaviour, cognitive impairments and hypolocomotion in mice. CUMS induced insulin resistance and hypercorticosteronemia in mice. CUMS and diabetes impaired insulin signaling pathway, ATG and muscarinic cholinergic system in the hippocampus. In addition, CUMS impaired Smoothened (Smo)-Glioma associated oncogene-1 (Gli1) pathway in the hippocampus. CUMS and diabetes downregulated muscarinic cholinergic receptor-4 (mAChR4) expression in striatum but not in hippocampus. Both CUMS and diabetes were associated with oxidative stress, inflammation and apoptosis. Chronic UD treatment (50 mg/kg, p.o.) significantly reverted CUMS and diabetes mediated cognitive impairment, depressive like behaviour and insulin resistance. UD reduced hypercorticosteronemia in stressed mice. Chronic UD administration modulated insulin signaling pathway, ATG and muscarinic cholinergic system in the hippocampus of chronically stressed and diabetic mice. Chronic UD administration effectively modulated hippocampal Smo-Gli1 pathway in stressed mice. UD treatment significantly reduced hyperglycemia, body weight loss and polydypsia in diabetic mice. UD administration significantly ameliorated hippocampal glucose transporter-4 (GLUT4) membrane translocation in diabetic but not in stressed mice. UD administration did not modulate mAChR4 expression in striatum and hypolocomotion. Chronic UD administration attenuated oxidative stress, inflammation and apoptosis in stressed and diabetic mice. These results suggest that chronic administration of UD extract might prove to be effective for depression and diabetes related neurological disorders.

Introduction

Depression is a mood disorder in which feelings of sadness, anger or frustration interfere with the daily life for weeks or longer. The symptoms of depression includes agitation, restlessness and anger, irritability, becoming isolated, fatigue and lack of energy, hopeless and helpless feeling, worthless, self-hate, loss of interest or pleasure in activities that were once enjoyed, sleep-wake abnormalities, thoughts of death or suicide etc [1].

Depression is associated with the hyperactivity of the hypothalamic-pituitary-adrenal axis (HPA axis) [2-3]. The hormone cortisol is the end product of activation of the HPA axis. It has been reported that uncontrollable stressors likely to be associated with elevations in cortisol level [3]. Hyperactivation of HPA axis is known to induce neurodegenerative process, reduce neurogenesis and cognitive dysfunction [4-5].

Hyperglycemia is associated with oxidative stress in hippocampus, resulting in neurological disorders such as memory impairment, depression and anxiety [6-7]. Hypercorticosteronemia induces over production of reactive oxygen species and elevation of cytosolic calcium, and subsequent increase in the calcium dependent death in neuronal cells [8]. Chronic activation of the HPA axis lead to condition called glucocorticoid resistance where immune cells are no longer able to assess cortisol signal, thus leading to increase in both cortisol level and inflammation [3]. Reactive nitrogen species such as nitric oxide (NO) has been implicated in stress mediated inflammation and cognitive deficit [9]. In addition, NO impairs autophagy (ATG) in rat primary cortical neurons which is known to modulate neuronal health [10].

The hippocampus region of brain that regulates learning and memory processes is vulnerable to oxidative stress and hypercortisolemia [11-13], during the depressive episodes [14]. Hippocampal muscarinic receptors are known to modulate cognition. Thus, hippocampal cholinergic dysfunctions might underlie depression associated cognitive impairments [14]. Depression dysregulate brain derived neurotrophic factor (BDNF) and mitogen activated protein kinase (MAPK) levels, which is known for its modulation in synaptic plasticity [15-16]. Sonic hedgehog (Shh), a mitogenic protein, has been implicated in neurological disorders. It has been demonstrated that, dysregulation of Shh co-receptors like patched (Ptch) and Smo within the dentate gyrus subfield of the hippocampus is associated with depressive phenotype [17].

Diabetes mellitus, commonly referred to as diabetes, is a chronic disease in which the body cannot regulate the amount of sugar in the blood. Diabetes can be caused by low insulin, resistance to insulin, or both [18-19]. Prolonged hyperglycemia often associated with nerve damage [20]. In the central nervous system, diabetes exacerbates depression, phobias,

anorexia and cognitive deficit [21-22]. Diabetes induces oxidative stress and inflammation in the hippocampal neurons resulting in neurodegeneration [6, 23]. Animal models also revealed the persistence of depression and neurocognitive impairment during chronic diabetes. Streptozotocin (STZ) induced diabetes showed depressive like behaviour [24-25] and cognitive deficit in rodents [26].

Dysfunctioning of neuronal peroxisome proliferator-activated receptor-γ (PPARγ) was observed in cognitive dysfunction [27]. A recent study reported that downregulation of PPARγ levels in the hippocampus of diabetic mice is associated with depressive like behaviour in forced swim test (FST) [28]. Diabetic neuropathies in brain are induced by the deficiency of BDNF [29].

Diabetes induces oxidative stress and inflammation in hippocampus [6]. Chronic diabetes is known to elevate the level of NO. NO induces inflammation of hippocampal neurons [30]. NO derived from inducible form of nitric oxide synthase (iNOS) contribute to the depressive like behaviour in mice due to cortical damage [9]. Chronic diabetes induced cholinergic hypofunction resulting in cognitive deficit [31-32]. Chronic diabetes resulted in motor activity deficit [33].

St. John's wort also known as *Hypericum perforatum* (HYP) is a flowering plant, belonging to the family Hypericaceae [34-35]. Clinical trials revealed that HYP extract is superior for depressive disorder and their effectiveness is similar to that of standard antidepressants with fewer side effects [36]. HYP extract improves spatial memory [37], proliferation of progenitor cells and dendritic spine, restored the synaptic plasticity [38] and Alzheimer's pathology [39]. St. John's wort and its active constituent hyperforin protect rat as well as human islets against cytokine mediated β-cell injury in type 1 diabetes [40].

In randomized controlled trial, fluoxetine (FLX) exerted neurocognitive improvement in patients with moderate depression [41]. FLX treatment reduced the level of glycated hemoglobin during co-mobidity depression and diabetes in rodents. FLX ameliorated spatial learning and showed hypoglycaemic and antidepressant effects during CUMS associated diabetes [42].

Rosiglitazone (ROSI) is a thiazolidinedione class of oral antidiabetic drug for therapy of type 2 diabetes mellitus [43-44]. ROSI increases glucose transport in adipose tissue and muscle by enhancing synthesis and translocation of glucose transporters [45-46]. ROSI improves cognition and memory performance in patients with mild Alzheimer disease and animal models of Alzheimer disease [47]. It has been reported that, insulin sensitizing drug ROSI exhibited significant declines in depression severity associated with insulin resistance [48].

Currently available antidepressants are known to improve neurocognitive functions depending on clinical, social and emotional factors [49]. Studies have shown that, therapy with antidepressants is correlated with an increased risk of suicidal behaviour in those aged under 25 [50]. Sexual side effects are also common with the use of fluoxetine, such as loss of sexual drive, failure to reach orgasm and erectile dysfunction [51]. Antidepressant medication increases the risk of hyperglycemia and diabetes mellitus. The incidence of diagnosed diabetes is higher among antidepressant users than nonusers [52-53]. Many currently available antidiabetic drugs are known to induce hypoglycaemia and associated cognitive impairment [54]. It has been reported that patients receiving chronic pioglitazone therapy showed increased incidence of bladder cancer compared to general population [55-56]. Chronic rosiglitazone therapy is associated with an increased incidence of myocardial infarction and heart failure in type 2 diabetic patients [57].

Urtica dioica (UD) is an herbaceous plant and commonly known as stinging nettle, belonging to the family Urticaceae [58-59]. Administration of UD leaves repaired pancreatic tissue damage in STZ induced diabetic rats [60]. UD administration significantly reduced hyperglycemia, insulin resistance and hyperlipidemia in fructose induced diabetic rats [61]. UD is known to reduce hypercorticosteronemia and depressive like behaviour in dexamethasone induced diabetes [62]. In clinical trial, UD is reported to have glycemic control in type 2 diabetic patients by lowering the levels of fasting and postprandial blood glucose [63]. Earlier studies reported that administration of diabetic patients with UD significantly increased antioxidant capacity and reduced inflammatory stress and glycated hemoglobin [64-65].

Objectives of the study

- To investigate the effect of *Urtica dioica* (stinging nettle) extract against chronic unpredictable stress (CUMS) induced depression and its associated complications in mice.
- To evaluate the effect of *Urtica dioica* extract on streptozotocin induced type 2 diabetes and its associated complications in mice.

Materials and methods

UD leaves and HYP was collected from the North Western Himalayan region and authenticated from Department of Forest Product, Dr. Y.S. Parmar University of Horticulture & Forestry, INDIA. Leaves of UD and aerial parts of HYP were air-dried under shade, pulverized and passed through 40 mesh sieves followed by extraction and phytochemical analysis. Standardization of plant materials was performed [66]. Identification of active

constituents in UD leaves extract was performed by liquid chromatography-mass spectrometry (LC-MS) analysis [67]. Identification of hyperforin and hypericin in St. John's wort extract was performed by high-performance liquid chromatography (HPLC) [68] and LC-MS analysis [69].

Male Swiss albino mice (aged 8-10 weeks) weighing 25-30 g was used in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines.

To study the effect of depression as a risk factor for diabetes and associated behavioural dysfunction and the effect of UD and reference standard drugs, animals were divided into groups and treated as follows: group I received 0.3% caboxymethyl-cellulose (0.3% CMC) and served as control; group II was exposed to CUMS and received vehicle (0.3% CMC); group III was subjected to CUMS and received FLX (10 mg/kg); group IV received FLX (10 mg/kg); group V was subjected to CUMS and received ROSI (5 mg/kg); group VI received ROSI (5 mg/kg); group VII was subjected to CUMS and received HYP extract (350 mg/kg); group VIII received HYP extract (350 mg/kg); group IX was subjected to CUMS and received UD extract (50 mg/kg); group X received UD extract (50 mg/kg). All drugs used in the study were administered by gastrointestinal gavage (p.o.). Drugs were administered between 5:00 p.m. and 7:00 p.m. once a day for 21 consecutive days. Dose of St John's wort, UD, FLX and ROSI used in the present experiment were selected from previous results [37, 70-72].

The animals were subjected to CUMS paradigm as described previously [73-74] with some modifications. Animals undergo stress paradigm once a day over a period of 21 days as follows: day 1, cold swim (8°C, 3 min); day 2, tail pinch (1 min); day 3, food and water deprivation (24 h); day 4, immobilization (3 h); day 5, overnight illumination; day 6, foot shock (20 trials, 0.5 mA, 5.0 sec maximum duration, 1 min intervals); day 7, tail pinch (2 min); day 8, cold swim (10°C, 5 min); day 9, overnight illumination; day 10, foot shock (20 trials, 0.5 mA, 5.0 sec maximum duration, 30 s intervals); day 11, food and water deprivation (24 h); day 12, immobilization (4 h); day 13, tail pinch (3 min); day 14, overnight illumination with wet cage; day 15, cold swim (6°C, 3 min); day 16, foot shock (20 trials, 0.5 mA, 5.0 sec maximum duration, 1 min intervals); day 17, food and water deprivation (24 h); day 18, tail pinch (2 min); day 19, immobilization (5 h); day 20, overnight illumination with tilted cage; day 21, cold swim (8°C, 3 min).

To study the effect of UD on STZ induced diabetes and associated neurological complications, animals were divided into two groups, viz., G1 – normal control and the rest

of the animals were administered with STZ (50 mg/kg, i.p.) for five consecutive days. The animals which showed the blood glucose level ≥200 mg/dl were considered for the study. Animals did not show hyperglycemia was removed from the study. The diabetic animals were then randomly divided into three groups, viz., G2 – STZ, G3 – STZ + hydro-alcoholic extract of UD (50 mg/kg) and G4 – STZ + ROSI (5 mg/kg). Hydro-alcoholic extract of UD or ROSI or vehicle (0.3% CMC in water for injection) was administered through oral gavage once daily from the 6th day after STZ injection till 60th day.

After drugs treatment animals were submitted to different behavioural studies to assess depressive like behaviour in forced swim test [75], tail suspension test (TST) [76], sucrose preference test [77]. Cognitive performance was evaluated by Morris water maze task (MWM) and passive avoidance step-through task (PA task) [78]. Motor functions were assessed using actophotometer [79] and rota-rod [80] apparatus.

Animals were sacrificed by cervical dislocation. Hippocampus and striatum were isolated and total RNA was isolated using Trizol reagent. Real-time quantitative reverse transcription PCR was used to study mRNA expression. Western blot analysis was performed for protein expression. Immunofluroscence (TNF-α) and histopathology studies were performed on 4 micron sections of hippocampus.

Blood glucose levels were measured using Accu-check blood glucose monitoring system. The levels of serum insulin were determined by chemiluminescent immunoassay kit. An HPLC-UV system was used for quantification of plasma corticosterone, using dexamethasone as an internal standard [81]. The level of plasma thiobarbituric acid reactive substances [82], NO [62], catalase [7] and total thiol [83] were determined. *In vitro* assays on hippocampal slices were performed to evaluate the effect of UD on Smo-Gli pathway and GLUT4 membrane translocation. Identification of hyperforin, hypericin and scopoletin on brain homogenate of stressed mice was performed by HPLC-UV system. The statistical significance was assessed by one-way analysis of variance followed by Tukey's post-hoc test using Graphpad Prism.

Results

Standardization of plant materials

UD showed total ash 21.2 %w/w, acid insoluble ash 3.04 %w/w and water insoluble ash 3.3 %w/w. UD showed water soluble extractives 23.3 %w/w and alcohol soluble extractives 25.3 %w/w. We also observed the presence of 5-hydroxytryptamine (5HT) and scopoletin during phytochemical analysis. In LC-MS analysis, UD leaves extract showed presence of scopoletin, gentisic acid, esculetin, quercetin and rutin. In HPLC analysis, the crude hydro-

alcoholic extract of UD leaves showed presence of scopoletin. HYP showed water soluble extractives 14.2 %w/w and alcohol soluble extractives 14.02 %w/w. Moisture content in UD leaves was 7.28 % and HYP was 3.72 %. HYP showed total ash 6.2 %w/w, acid insoluble ash 2.4 %w/w and water insoluble ash 3.2 %w/w. Negative ion LC-MS as well as HPLC chromatograms of St. John's wort showed the presence of hyperforin and hypericin. Phytochemical evaluation on UD and HYP extract revealed the presence of alkaloids, carbohydrates, glycosides, phenolics, flavonoids, proteins and amino acids.

Effect of UD extract on stress induced depression and associated complications

Chronic stress significantly increased the duration of immobility in FST and TST. Chronic UD treatment significantly improved the mobility period in FST and TST comparable to FLX, ROSI and HYP treatment. Chronic stress significantly reduced sucrose preference from week two onwards. UD treatment significantly ameliorated sucrose preference from second week as compared to ROSI and FLX. HYP treatment did not modulate sucrose preference on week two however showed improvement on week three.

On day 1 of training, all the animals showed similar learning behaviour in Morris water maze task. The control animals showed improvement in learning between trials from day 23-25 as evident from decrease in the escape latency, while chronically stressed mice did not show any significant improvement in learning between the trials. Chronic UD treatment significantly decreased the escape latency on day 3 and day 4 in stressed animals comparable to chronic FLX, ROSI and HYP treatment. In the probe trial, number of crossings across the platform area was significantly decreased in stressed mice, while chronic UD treatment significantly increased the crossings comparable to chronic ROSI, HYP and FLX treatment. In PA task, CUMS decreased the step through latency (STL) during memory retention trial on week three. Chronic UD treatment significantly ameliorated STL in stressed animals comparable to FLX, ROSI and HYP treatment. Chronic UD treatment significantly improved STL from week two onwards in control animals as well.

CUMS exposure significantly reduced falling latency after week two onwards elicited muscle coordination deficit in rotarod test. Locomotor activity in actophotometer test was significantly decreased in chronically stressed mice on week three. Neither UD nor reference standard drugs modulated motor deficit in stressed mice.

In the present study, chronic stress or drugs treatment did not modulate the level of fasting blood glucose and serum insulin level. CUMS significantly induced depression mediated insulin resistance and hypercorticosteronemia. Chronic UD and ROSI treatment significantly reduced depression mediated insulin resistance, while FLX and HYP treatment did not

modulate insulin resistance. UD treatment significantly attenuated hypercorticosteronemia in stressed mice comparable to chronic FLX, ROSI and HYP administration.

Effect of UD extract on depression mediated alteration in hippocampal insulin signaling

CUMS significantly downregulated the expression level of PPARγ, insulin receptor (IR), insulin receptor substrate-1 (IRS1), insulin receptor substrate-1 (IRS2), phosphatidylinositide 3-kinase (PI3K), protein kinase-B (PKB), GLUT4, INSG1 and MAPK1 mRNA in the hippocampus region of brain. Chronic UD treatment significantly reversed stress mediated downregulation in the level of PPARγ, IR, IRS1, PI3K, PKB, GLUT4, insulin induced gene 1 (INSG1) and MAPK1 mRNA which is comparable to ROSI administration. UD treatment did not modulate the level of IRS2 in stressed mice. Chronic UD and HYP treatment significantly ameliorated MAPK1 mRNA in control animals as well. Chronic FLX and HYP administration significantly upregulated the mRNA expression of MAPK1 in stressed animals. Further, chronic stress or drugs treatment did not modulate the expression level of hippocampal insulin like growth factor 1 receptor (ILGF 1r) and glucagon-like peptide-1 (GLP1) mRNA. Chronic FLX and HYP administration did not regulated the mRNA involved in insulin signaling pathway. Chronic stress significantly downregulated the GLUT4 membrane translocation in hippocampus. Chronic ROSI administration significantly ameliorated GLUT4 membrane translocation in hippocampus, while UD administration did not modulate it.

Effect of UD extract on depression mediated impairment in Smo-Gli pathway

In the present study CUMS did not modulate the expression level of hippocampal Shh, Ptch1 and hedgehog interacting protein (hHIP) mRNA. Neither UD nor standard drugs modulated the level of Shh and Ptch1, while chronic HYP administration significantly downregulated the level of hHIP mRNA in stressed mice. CUMS significantly downregulated the mRNA expression of Smo, Gli1, cyclin D1, BDNF and tyrosine receptor kinase-B (TrkB) in hippocampus. Chronic UD treatment significantly upregulated the level of Smo, Gli1, cyclin D1 and TrkB in stressed mice comparable to chronic HYP treatment. UD administration did not modulate the BDNF mRNA level in stressed mice. UD and HYP administration significantly upregulated the mRNA level of Ptch1, Smo and Gli1 in control animals as well. FLX significantly ameliorated BDNF and TrkB mRNA level in stressed animals. ROSI upregulated TrkB mRNA but not BDNF level in stressed mice. FLX and ROSI administration did not modulate the level of Smo, Gli1 and cyclin D1 mRNA in stressed or control animals. *In vitro* assays on hippocampal slices revealed that, UD treatment upregulated the Gli1 and Ptch1 mRNA expression, and the effect of UD was blocked by

pretreatment of slices with Smo antagonist cyclopamine. Herein, HYP treatment produced action similar to that of UD treatment.

Depression mediated alteration in hippocampal and striatal cholinergic system and the effect of UD extract

While assessing the effect of depression-mediated alteration in hippocampal and striatal cholinergic system and the effect of UD and FLX, we observed that CUMS significantly downregulated the level of muscarinic acetylcholine receptor-1 (mAChR1) in hippocampus, while upregulated the mRNA and protein levels of mAChR4 in striatum. Chronic UD administration significantly upregulated the mAChR1 expression in hippocampus, while did not modulate striatal mAChR4 level in stressed mice comparable to FLX administration. Neither CUMS nor drugs administration modulate the level of mAChR1 in striatum and mAChR4 in hippocampus. Chronic HYP and ROSI administration did not modulate the mRNA level of hippocampal mAChR1 and striatal mAChR4 in stressed mice. Chronic stress significantly upregulated the expression level of hippocampal AChE and downregulated choline acetyltransferase (ChAT) protein in hippocampus, which were significantly reversed by chronic UD administration. Further, chronic FLX administration downregulated hippocampal acetylcholinesterase (AChE) protein but did not modulate ChAT level in hippocampus.

Effect of UD extract on CUMS induced oxidative stress, inflammation and neuronal damage

We observed that CUMS significantly downregulated the level of B-cell lymphoma 2 (BCL2), apoptosis inhibitory protein-2 (AIP2), ATG5 and ATG7 mRNA in hippocampus. Chronic UD administration significantly reversed stress induced decline in BCL2 mRNA comparable to standard drugs. UD treatment significantly upregulated ATG5 and ATG7 mRNA in stressed mice comparable to FLX and HYP treatment. ROSI treatment did not modulate ATG mRNA expression in stressed mice. Chronic FLX and HYP administration significantly upregulated AIP2 expression, while UD and ROSI did not modulate it. Chronic stress significantly upregulated inflammatory biomarkers like iNOS, interleukin-6 (IL6) and tumor necrosis factor alpha (TNFα) mRNA in hippocampus, which were significantly reversed by chronic UD, FLX, HYP and ROSI administration. CUMS induced TNFα expression and neuronal damage in hippocampal sections were alleviated by chronic UD, ROSI, HYP and FLX administration. CUMS significantly increased the level of thiobarbituric acid reactive substances (TBARS) and NO, while reduced the level of catalase and total thiol in plasma. FLX, ROSI and HYP administration significantly reduced the level

of TBARS, while UD did not reduce TBARS level in plasma. Chronic UD administration significantly reversed stress induced alteration in the level of NO, catalase and total thiol in plasma comparable to chronic ROSI, HYP and FLX administration.

We also observed the presence of scopoletin (stinging nettle) and St. John's wort constituents (hyperforin & hypericin) in brain after CUMS paradigm.

Effect of UD extract on STZ induced chronic diabetes and its associated depressive like behaviour and cognitive dysfunction

We evaluated the effect of UD extract on STZ induced hyperglycemia, hypoinsulinemia and insulin resistance in mice. Herein, we observed that STZ treatment significantly elevated the level of fasting and postprandial blood glucose, while decreased the level of serum insulin, which were significantly reversed by chronic UD and ROSI administration. After STZ injection, animals exhibited decreased body weight as compared with control mice. Chronic treatment with UD and ROSI to diabetic mice after 5th–60th day significantly ameliorated body weight loss. In our study, long standing diabetes significantly increased the water intake, which is a classic sign of diabetes. Chronic administration of UD and ROSI to diabetic mice significantly reduced the water intake.

STZ induced diabetic mice showed significant increase in the duration of immobility in FST and TST. Chronic UD administration significantly reduced the duration of immobility in diabetic mice comparable to chronic ROSI administration. STZ induced diabetes significantly reduced the locomotor activity in actophotometer test and motor coordination activity in rotarod test. Neither UD nor ROSI administration attenuated motor function deficit in diabetic mice. In Morris water maze task, diabetic mice showed significantly increased in escape latency. The control animals showed significant improvement in learning between trials evident from the decrease in the escape latency. The number of crossings across the platform area was also significantly decreased in diabetic mice. UD and ROSI treatment significantly decreased the escape latency, whereas improved the number of crossings across the platform area (probe trial). In PA task, diabetic mice showed no alteration in STL on memory retention trial when compared with their acquisition trail STL. During memory retention trial the diabetic animals showed decreased STL as compared to normal animals. Further, chronic treatment with UD and ROSI significantly increased the STL during memory retention trial compared with STZ induced diabetic mice.

Effect of UD extract on diabetes mediated alteration in hippocampal insulin signaling

In the present study long standing diabetes significantly downregulated the expression level of IR, IRS1, IRS2, PI3K, PKB, GLUT4, INSG1 and MAPK1 mRNA in hippocampus.

Chronic diabetes did not modulate the mRNA expression of hippocampal PPARγ, ILGF 1r and GLP1. Chronic UD and ROSI administration significantly upregulated the mRNA expression of hippocampal PPARγ, IR, IRS2, PI3K, PKB, GLUT4, INSG1 and MAPK1 in diabetic mice. Chronic ROSI administration significantly upregulated hippocampal IRS1 mRNA, while UD administration did not modulate it. Neither chronic diabetes nor UD or ROSI administration modulated the mRNA level of ILGF 1r and GLP1 in hippocampus. STZ induced diabetic mice showed significant downregulation in the level of cytosolic and membrane GLUT4 protein. Besides, chronic UD and ROSI treatment significantly increased the expression of cytosolic and membrane GLUT4 protein in diabetic animals. *In vitro* stimulation of hippocampal slices by UD and insulin increases the association of GLUT4 membrane protein, an effect that was blocked by the acute treatment with PI3kinase inhibitor LY294002.

Diabetes mediated alteration in hippocampal and striatal cholinergic system and the effect of UD extract

STZ induced diabetic mice showed significant downregulation in the hippocampal mAChR1 whereas upregulated striatal mAChR4 mRNA and protein expression. Chronic UD and ROSI treatment significantly increased the mRNA and protein expression of hippocampal mAChR1, while did not modulate the striatal mAChR4 in diabetic mice. Neither chronic diabetes nor UD or ROSI administration modulated the mRNA expression of mAChR1 in striatum and mAChR4 in hippocampus. Chronic diabetes significantly upregulated the protein expression of AChE whereas downregulated ChAT expression in hippocampus, which were significantly reversed by chronic UD administration. Chronic ROSI administration significantly upregulated the protein expression of hippocampal ChAT but did not modulate the level of AChE in diabetic mice.

Effect of UD extract on diabetes mediated oxidative stress, inflammation and neuronal damage

BDNF, TrkB and cyclin D1 mRNA expression was significantly downregulated in the hippocampus of chronically diabetic mice. Chronic UD and ROSI administration significantly increased the mRNA expression of BDNF, TrkB and cyclin D1 in diabetic mice. STZ induced diabetic mice significantly downregulated the mRNA expression of BCL2 and ATG7 whereas upregulated iNOS and TNFα in the hippocampus, which were significantly reversed by chronic UD and ROSI administration. Chronic diabetes did not modulate the mRNA expression of AIP2, ATG5 and IL6 in the hippocampus. Chronic UD and ROSI administration significantly increased the mRNA expression of hippocampal ATG5 in

diabetic mice. Chronic UD and ROSI administration did not modulate the mRNA expression of AIP2 and IL6 in the hippocampus of chronically diabetic mice. STZ induced TNFα expression and neuronal damage in hippocampal sections were alleviated by chronic UD and ROSI administration. The level of plasma TBARS and NO were significantly increased whereas catalase and total thiol were decreased in diabetic animals, which were significantly reversed by chronic UD and ROSI administration.

Discussion

Anhedonia, a disability to experience pleasures, is a major symptom of depression [84]. It has been reported that hedonic deficits can also be induced by chronic unpredictable stress in rodents [85-86]. Using behavioural animal models, it was observed that anhedonia is associated with increased floating during forced swimming and decreased novel object exploration [85]. In the present study, sucrose consumption was significantly declined from week 2 onwards in stressed mice. Exposure of mice to CUMS resulted in increased duration of immobility in FST and TST, indicating depressive like behaviour. While, depressive like behaviour in stressed mice was attenuated by administration with FLX, ROSI, HYP and UD. Current study confirms and extends previous findings, in which UD extract has antidepressant like property [62, 87]. HYP is known to possess potent antidepressant property [37, 88]. FLX reduced hedonic deficit and increased mobility in FST resulted in antidepressant action [89]. ROSI is known to induce neurogenesis and attenuate depressive like behaviour in stressed animals [90].

Clinically, stress or depression has been reported to induce neurocognitive dysfunctions [91]. In line with the literature, we observed that CUMS increased the escape latency in MWM task. This increase in escape latency is a reliable index of spatial memory dysfunction in stressed mice [92]. In addition, we observed that CUMS-induced depression led to associative memory dysfunction in mice evident from decreased step through latency in PA task on week three. Chronic administration of UD, FLX, HYP and ROSI to chronically stressed mice significantly reduced the escape latency in MWM task and ameliorated number of crossings in probe trials. Further, UD, FLX, HYP and ROSI treatment significantly reversed associative memory dysfunction associated with depressive like behaviour. Interestingly, chronic UD treatment significantly ameliorated associative memory in control animals as well from week two onwards. UD is known to reduce learning and memory deficits in hypercorticosteronemic mice [62]. St John's Wort was found to have a novel type of antidepressant with memory enhancing properties [37, 93]. FLX attenuated impaired cognition induced by chronic mild stress in MWM task [94]. Chronic unpredictable stress is

known to induce motor function deficit [85, 95]. Chronically depressed mice showed defective muscle coordination in rota rod test from week two onwards. Locomotor activity in depressed mice was reduced on week three. Chronic UD, FLX, HYP and ROSI administration to depressed or normal mice showed no alteration in muscle coordination and locomotor activity.

Clinically, depression has been reported to induce type 2 diabetes mellitus [96]. In animal models, CUMS exhibited insulin resistance, hypoinsulinemia and hyperglycemia [97-98]. In the present study, 21 day of CUMS significantly induced insulin resistance in mice. However the level of fasting blood glucose and serum insulin was not significantly altered. Chronic UD and ROSI administration significantly reduced insulin resistance in stressed mice, while FLX and HYP did not modulate insulin resistance. Current follow-up study confirms and extends previous findings, in which UD extract has shown antidiabetic property [60, 70].

It has been reported that, chronic supraphysiological level glucocorticoids induces depressogenic-like effect and hyperglycemia in animal models [62, 99]. In addition, excess of glucocorticoids impaired the insulin action and glucose homeostasis in hippocampal neurons, resulting in disturbed synaptic plasticity [100]. In the present study we observed that, CUMS significantly increased the level of plasma corticosterone and induced memory deficit in mice. Chronic UD, FLX, ROSI and HYP treatment significantly reversed stress-induced hypercorticosteronemia in mice. It has been demonstrated that UD extract attenuated hypercorticosteronemia and hyperglycemia in dexamethasone induced behavioural deficits [62]. ROSI is known to improve uptake of glucose in 3T3-L1 adipocyte with insulin resistance induced by hypercorticosteronemia [101].

Accumulated evidences suggest that, insulin signaling pathway is important not only for metabolic process or neurotransmitter homeostasis in the hippocampus but also for proper synaptic plasticity, energy metabolism, neuroprotection and cognitive function [102-103]. We observed that, CUMS-induced depressed mice have insulin resistance which might be due to hypercorticosteronemia or downregulation of IR. Both hypercorticosteronemia or downregulation of IR led to dysregulation of insulin signaling components like IRS1, IRS2, PI3K, PKB, MAPK1, INSG1 and GLUT4, resulting in defective neuroprotection, neuronal development and memory formation [104-105]. Hypercorticosteronemia and diabetes are associated with downregulation of PPARγ expression in hippocampus and hypothalamus, which also contribute to the cognitive impairment [106]. Herein we observed that, CUMS-induced depression with comorbid insulin resistance significantly downregulated the mRNA expressions of PPARγ, IR, IRS1, IRS2, PI3K, GLUT4, PKB, MAPK1 and INSG1 in the

hippocampus under non-fasting condition. In the present study, chronic UD treatment significantly upregulated the mRNA expression of hippocampal PPARγ and restored stress-induced alterations in insulin signaling pathway, which was comparable to chronic ROSI administration. However, UD had no effect on the level of hippocampal IRS2 mRNA after CUMS procedure. Chronic FLX and HYP treatment did not modulate hippocampal insulin signaling cascade in depressed and non-depressed mice, while ameliorated MAPK1 mRNA expression in stressed mice. Chronic UD and ROSI administration did not modulate insulin signaling cascade in control animals. However, UD and ROSI were associated with upregulation of MAPK1 mRNA expression in control animals. The coumarin, scopoletin in UD [67] increases the gene expression of PPARγ and reduces insulin resistance [107]. Recent studies suggested that PPARγ agonist ROSI improves learning and memory in both human and animal models [108-109].

In the central nervous system, the level of GLUT4 was reduced during depression and diabetes [62, 110]. Herein we observed that, CUMS-induced depressed mice with insulin resistance showed reduced translocation of GLUT4 protein to the hippocampal plasma membrane. Chronic UD administration did not induce GLUT4 membrane translocation, while ROSI administration ameliorated hippocampal GLUT4 translocation in depressed mice. Amelioration of GLUT4 membrane translocation in hippocampal neurons improves neurocognition [111].

Chronic stress is known to affect hippocampal neurogenesis precipitating cognitive impairment [112]. Shh, a regulator of adult hippocampal neurogenesis is essential for the maintenance of the adult neurogenic niches [113]. In the present study CUMS, FLX, ROSI, HYP and UD treatment did not modulate the mRNA expression of Shh in hippocampus. Also, the level of Shh co-receptor Ptch1 was not modulated by chronic FLX and ROSI administration. The level of Ptch1 mRNA expression was ameliorated by chronic HYP and UD administration in control animals. Chronic UD and ROSI administration did not modulate mRNA expression of Ptch1 in depressed animals. It was observed that CUMS reduced the mRNA expression of Smo and Gli1 in the hippocampus. Gli1 is a target gene of Shh signaling pathway. Chronic UD and HYP administration led to significant improvement in Smo and Gli1 mRNA expression in both depressed and non-depressed mice. Chronic FLX and ROSI administration did not modulate Smo and Gli1 mRNA expression in control and depressed mice. Only HYP administered depressed mice showed downregulation of hippocampal Hhip mRNA, a negative regulator of Shh signaling. A recent in vitro study suggests that depletion of both norepinephrine (NE) & 5HT, a clinical condition of

depression resulted in the modulation of Shh signaling but not in the case of selective depletion [17]. We report, CUMS, HYP or UD treatment did not modulate canonical Shh signaling. CUMS dysregulate non-canonical Shh signaling in hippocampus as observed through downregulation of Smo and Gli mRNA. It has been reported that hedgehog signaling is activated by non-canonical Src kinase pathway [114]. Further, activation of Src-MAPK pathway induces neuronal survival, neuronal repair and regeneration in hippocampus [105]. We observed that chronic UD and HYP administration significantly increased the mRNA expression of MAPK1 in control and stressed mice. We observed that, pretreatment of hippocampus slices with cyclopamine significantly downregulated the mRNA expression of Gli1 and Ptch1. Neither UD nor HYP could reverse cyclopamine induced downregulation of Gli1 and Ptch1 mRNA in hippocampal slices when compared with normal and purmorphamine groups, suggests that UD and HYP treatment modulates Smo-Gli pathway.

BDNF is reported to strongly influence synaptic morphogenesis, neuronal survival, long-term potentiation of brain synapses as well as adult hippocampal neurogenesis [115-116]. In the present study, CUMS significantly decreased the mRNA expression of BDNF, its receptor TrkB in hippocampus. Chronic UD, ROSI, FLX and HYP treatment normalized the mRNA expression of TrkB in stressed mice, while UD and ROSI treatment did not modulate the mRNA expression of BDNF in hippocampus. It was reported that activation of MAPK further mediate the expression of cyclin D1[117-118]. Cyclin D1 is co-localized with newly generated neurons in murine hippocampus [119]. Herein we observed that, cyclin D1 mRNA expression was downregulated, while chronic HYP and UD treatment significantly upregulated cyclin D1 mRNA expression in stressed mice.

A decrease in the ChAT expression was noted in the dorsal hippocampus of stressed mice [120]. Earlier reports suggest that CUMS increases AChE expression as well as activity in the hippocampus resulting into cognitive impairments [30, 121-122]. In the present study, CUMS resulted in upregulation of AChE whereas downregulation of ChAT protein expression in hippocampus. Chronic FLX treatment did not modulate ChAT expression in the hippocampus, indicating that FLX might not have any effect on acetylcholine (ACh) biosynthesis in control and depressed mice. While, UD administration significantly elevated ChAT expression in stressed mice. Besides, chronic UD and FLX administration downregulated the level of hippocampal AChE in depressed mice. It could be speculated that, the modulation of CUMS mediated cognitive impairments by UD and FLX could be a result of potentiation of ACh action at the neuronal junction of hippocampus. Both scopoletin and rutin have been known to reduce AChE activity in the hippocampus [123-124]. We observed

that CUMS differentially altered the mRNA and protein levels of mAChR subtypes in hippocampus, in which mAChR1 subtype was downregulated whereas mAChR4 expression was not modulated in stressed mice. mAChR1 is known to be involved in cognitive function and neurogenesis [125]. In the present study, chronic UD and FLX treatment significantly increased the expression of hippocampal mAChR1 in stressed mice, but UD and FLX *per se* did not modulate mAChR1 level. Chronic HYP and ROSI did not modulate mAChR1 mRNA expression in hippocampus. Chronic UD, FLX, ROSI and HYP treatment had no effect on hippocampal mAChR4 expression in control and stressed mice. Besides, mAChR4 mRNA and protein expression was significantly upregulated, while mAChR1 mRNA was unaffected in the striatum of chronically stressed mice. Chronic FLX, ROSI, HYP and UD treatment did not modulate mRNA expression of striatal mAChR1 and mAChR4 in depressed or non-depressed mice. UD and FLX did not reduce upregulation of striatal mAChR4 protein in depressed mice.

Striatum is densely innervated by cholinergic interneurons, co-expressed with dopaminergic D1 receptor that is crucial for motor behaviour [126]. Activation of mAChR4 in the striatum inhibits D1 receptor-induced locomotor stimulation in mice [127]. Our observations are consistent with previous study suggesting that major depression results in motor control system deficit [128]. Hence, it could be speculated that, decreased locomotor activity in depressed mice might be a result of enhanced striatal mAChR4 expression.

Chronic exposure to unpredictable stressors induces oxidative stress, inflammation and apoptosis, which further exacerbates the dysfunctioning of cholinergic neurons in hippocampus, resulting in cognitive impairments [30, 129]. Social stress increases the number of apoptotic cells in the hippocampus and temporal cortex [130], whereas antidepressants upregulate BCl2 expression and improve neural plasticity [131]. The ability of AIP2 to inhibit caspase-2 indicates that it occupies a unique position in programmed cell death initiated by endoplasmic reticulum stress [132]. In the present study CUMS significantly downregulated the level of hippocampal BCl2 and AIP2 mRNA. Besides, chronic UD, FLX, ROSI and HYP treatment significantly upregulated hippocampal BCL2 in stressed mice. In addition, chronic FLX and HYP administration ameliorated AIP2 mRNA in stressed mice, while UD and ROSI did not modulate it. CUMS decreased the level of ATG5 and ATG7 mRNA in hippocampus. Report suggests that dysfunctions in autophagy develop neuronal damage accompanied by ubiquitinated protein aggregates, indicating that basal levels of autophagy are essential for neuronal health [133]. In the present study chronic UD,

FLX, HYP but not ROSI ameliorated the level of ATG5 & ATG7 in chronically stressed mice.

CUMS plays an important role in dysregulation of monoaminergic-serotoninergic system in CNS with the oxidative load [134]. In the present study, CUMS resulted in elevation in the level of oxidative and nitrative stress markers in plasma, as indicated by raise in the levels of TBARS and NO, and depletion of antioxidants such as catalase and total thiol. Chronic treatment with UD, ROSI, FLX and HYP reversed CUMS-induced alteration in the level of catalase along with attenuation of oxidative and nitrative stress. However, chronic UD treatment did not significantly reverse the level of plasma TBARS in stresses mice.

Oxidative insult is known to induce cytokine proteins including ILs and TNFs [135]. Increased expression of iNOS in hippocampus make significant contributions to CUMS induced depressive like behaviour [136]. Cytokines are known to mediate inflammatory processes observed during neurodegenerative diseases such as vascular dementia and Alzheimer's disease [135]. Herein, CUMS induced depressed mice showed significantly elevated level of inflammatory mediator's viz. iNOS, TNFα and IL6 in the hippocampus. Besides, chronic UD, FLX, ROSI and HYP administration reversed upregulation of cytokines production in depressed mice. We observed that chronic stress significantly increased the expression of TNF α protein and neuronal damage in hippocampal section and this effect was reversed by chronic UD administration. In earlier studies ROSI, FLX and HYP showed anti-oxidant and anti-inflammatory effects [137-139].

We also evaluated the effect of UD and ROSI extract against diabetes mediated neurological disorders in mice. Experimental evidences suggest that, multiple lower doses of STZ, which cause partial destruction of β-cells, can be used to produce type 2 diabetes [140]. It is also well defined that long standing diabetes leads to depression [21], dementia [141], and sexual dysfunction [142]. Polydypsia and body weight loss are clinical symptoms associated with diabetes in both experimental animals and patients [143]. Accordingly, in the present study, multiple lower doses of STZ treatment significantly elevated the fasting and postprandial blood glucose in experimental animals. We observed that STZ induced diabetes resulted in increased water intake and body weight loss determined from 1st week to 8th week as compared to normal animals. Further, it was observed that the circulating insulin level was reduced in STZ induced diabetic animals. Treatment with UD and ROSI significantly reversed the STZ induced alteration in bodyweight, water intake, blood glucose and serum insulin level. Current study confirms and extends previous findings, in which UD extract has anti-diabetic property [60, 70].

After STZ treatment, diabetic mice showed depressive like behaviour in TST and FST. Our results are consistent with previous study demonstrated that, STZ induced diabetes associated with depressive like behaviour [144-146]. Earlier reports have shown that STZ induced diabetes in rodent exhibit motor system control deficit [145, 147]. In the present study chronic UD and ROSI administration significantly reduced the depressive like behaviour but did not reverse motor deficit in diabetic mice. The chemical constituents of UD like scopoletin, rutin and quercetin is reported to have antidepressant like effect [148-149].

The current study demonstrated that long standing diabetes caused impairment in acquisition and retrieval processes (spatial memory), as observed by performance in the Morris water maze test, which is in line with previous reports [150]. It was also observed that STZ induced long standing diabetes leads to associative memory dysfunction in mice evident from decreased step through latency in PA task. In the present study, chronic treatment with either UD or ROSI significantly improved the spatial and associative memory dysfunction associated with long standing diabetes. In line with our current results it was reported that ROSI improves learning and memory in both human and animal models [109].

STZ induced diabetic mice have elevated level of blood glucose which might be due to hypoinsulinemia or insulin resistance. In both cases, the downstream insulin signaling components are impaired, alongside deficits in neuronal development and memory formation [104-105]. It was reported that the level of ILGF1 was downregulated in the hippocampus of chronically diabetic mice [151]. GLP1 protects hippocampal neurons against diabetes associated advanced glycation end product damage [152]. Herein we observed that STZ induced diabetes significantly downregulated the mRNA expression of IR, IRS1/2, PI3K, PKB, MAPK1, INSG1 and GLUT4 in the hippocampus. We did not observe any significant alteration in the mRNA expression of hippocampal PPARγ, GLP1 and ILGF 1r in diabetic mice. We observed that chronic treatment with UD or ROSI upregulated PPARγ gene expression and restored diabetes induced alteration in insulin signaling components viz., IR, IRS, PI3K, PKB, MAPK1, INSG1 and GLUT4 in hippocampus, while UD did not modulate the expression of IRS1 in diabetic mice. UD and ROSI did not regulate GLP1 and ILGF 1r in diabetic mice. Earlier study demonstrated that UD extract upregulated the expression level of GLUT4 gene as well as reduced hypercorticosteronemia and depressive like behaviour in dexamethasone induced diabetic mice [62]. We observed that, diabetes significantly downregulated the level of anti-apoptotic BCl2 gene in the hippocampus, whereas ROSI or UD treatment significantly reversed BCl2 downregulation. It has been demonstrated that, ROSI protects the neuronal cells against advanced glycation end products-induced injury via

its anti-apoptotic property, that seems to be mediated by PPARγ activation, and suggested a beneficial role of ROSI in the treatment of Alzheimer's disease [153]. Herein, UD extract upregulated hippocampal BCL2 expression in diabetic mice, which might be through upregulation of PPARγ. Further, esculetin is known to activate the MAPK and PI3K/Akt pathways [154].

In the present study, STZ induced diabetic mice showed reduced level of GLUT4 protein in the cytosolic fraction of hippocampus. Moreover, GLUT4 translocation to the hippocampal plasma membrane was significantly reduced in diabetic mice. Besides, chronic UD and ROSI treatment significantly restored diabetes mediated alteration in GLUT4 content in cytosol and their translocation to the hippocampal plasma membrane. Previous study demonstrated that, ROSI induces insulin-stimulated glucose uptake as a result of increased GLUT4 translocation to the plasma membrane [155]. Exposure of L6-GLUT4myc muscle cell with UD extract led to significant increase in GLUT4 content in plasma membranes [156]. In agreement with this, the active constituents of UD extract like scopoletin, rutin, quercetin and esculetin modulates the level of PPARγ gene [157-159], which is known to activate IR→IRS1/2→PI3K→Akt/PKB pathway and induces GLUT4 translocation in hippocampal neurons [105, 111, 160]. UD and insulin treatment increased plasma membrane association of GLUT4 in hippocampal slices, an effect that was blocked by the PI3K inhibitor LY294002.

In the present study, chronic diabetes resulted in downregulation of ChAT whereas upregulation of AChE expression in the hippocampus. UD treatment for 8 weeks reversed the diabetes mediated modulation in ChAT and AChE levels. Scopoletin inhibits AChE activity, potentiates acetylcholine release from synaptosomes, amplifies hippocampal long-term potentiation and attenuates anticholinergic- and age-impaired cognitive deficit [124]. Rutin has also been known to reduce AChE activity in the hippocampus [123]. Besides, ROSI effectively modulated ChAT expression but did not show significant effect against AChE level.

In the present study, chronic diabetes significantly reduced the expression of mAChR1 in hippocampus, which is in line with a previous study suggesting that STZ-induced diabetes significantly downregulated the expression of mAChR1 in hippocampus [161]. Besides, chronic UD and ROSI treatment significantly upregulated hippocampal mAChR1 expression in diabetic mice. Our results suggest that neurocognitive improvement by UD treatment might be due to the modulation of muscarinic cholinergic system in hippocampus. Quercetin modulates cholinergic neurotransmission in the hippocampus [162-163] and attenuates memory impairment and anxiogenic-like behaviour in rodents [162]. Chronic diabetes

significantly increased mAChR4 expression in striatum along with hypolocomotion in mice. Our observations are consistent with previous studies suggesting that diabetes results in motor control system deficit [33]. Both chronic UD and ROSI treatment could not reverse diabetes-mediated alteration in striatal mAChR4 expression and hypolocomotion. BDNF is well known to modulate neuroprotective effect in hippocampus [164]. Cyclin D1 is known to induce neural stem cells proliferation and differentiation in the rat brain [165]. Herein, we observed that STZ induced diabetes significantly downregulated the mRNA expression of BDNF, TrkB and cyclin D1 in the hippocampus. Chronic UD and ROSI administration significantly attenuated diabetes mediated alteration in BDNF, TrkB and cyclin D1 expression in the hippocampus.

Growing body of evidence demonstrate that, oxidative stress, inflammation and apoptosis plays a central role in the onset and subsequent neurological complications of diabetes [6, 166] including cognitive impairment [30, 167]. In the present study, chronic diabetes induced inflammation as evidenced by upregulation of TNFα and iNOS expression. Further, the level of ATG7 was significantly downregulated in chronically diabetic mice. Chronic diabetes did not modulate the level of AIP2, ATG5 and IL6 in the hippocampus. We observed that chronic diabetes significantly increased the expression of TNF α protein and neuronal damage in hippocampal section and this effect was reversed by chronic UD administration. STZ significantly elevated the level of TBARS and NO in plasma. The level of antioxidant catalase and total thiols were significantly decreased in diabetic mice. Chronic treatment with UD and ROSI significantly reversed diabetes-induced alteration in the level of antioxidants (catalase and total thiol) along with attenuation of oxidative stress, inflammation and apoptosis. UD and ROSI did not regulate the level of AIP2 and IL6 in the hippocampus of diabetic mice. UD extract has shown antioxidant and antiinflammatory effects in rodents [168].

Conclusion

In conclusion, UD modulated muscarinic cholinergic system, Smo-Gli pathway, insulin signaling and glucose homeostasis in hippocampus resulting in neurocognitive improvement during co-morbidity of depression and diabetes. In addition, UD presents a notable anti-inflammatory, antioxidant and anti-apoptotic effects, which appears to be related with the protection of hippocampal neurons during chronic stress and diabetes. Phytochemical analysis revealed the presence of flavonoids and phenolics in the UD extract, which might possibly involved in the anti-inflammatory, antioxidant and anti-apoptotic effects. UD extract might prove to be effective for the chronic stress and diabetes related neurological disorders.

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